Results of a Phase I Trial Using Recombinant Soluble Tumor Necrosis Factor Receptor (p80) Fusion Protein (sTNF-R:Fc) to Treat Rheumatoid Arthritis

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Tumor Necrosis Factor: Role in Rheumatoid Arthritis

In addition to its participation in the physiologic response to infection and neoplasm, local TNF-α expression may contribute to the pathogenesis of chronic inflammatory diseases, such as rheumatoid arthritis (RA).(1) TNF-α is a homo-trimer consisting of three identical 17 kilodalton polypeptide subunits. It is expressed primarily by stimulated mononuclear phagocytes. The best studied stimulus for TNF-α secretion is bacterial lipopolysaccaride (LPS). Increased levels of TNF-α are observed in many infectious, neoplastic and autoimmune diseases. In addition to its role in infection, TNF-α is also known to play a role in resistance to neoplasms.(2)

Evidence supporting a role for TNFα in the pathogenesis of RA includes: the presence of TNF-α at the cartilage-pannus junction in RA patients(3) and increased levels of TNF-α in RA synovial fluid.(3,4) Furthermore, TNF-α production is increased by the synovial cells of patients with active RA, but not in synovial cells from patients with inactive RA.

Several proinflammatory actions of TNF-α may contribute to its role in the pathogenesis of RA; in addition to stimulating the release of other proinflammatory cytokines, including interleukin-6 (IL-6), IL-8, IL-1ß and leukemia inhibitory factor (LIF), TNF-α also induces the release of proteases from neutrophils, fibroblasts and chondrocytes.(5-7) These enzymes, including collagenase and other neutral metalloproteinases, are likely to be responsible for joint destruction in RA. TNF-α also induces the expression of endothelial adhesion molecules (e.g. intracellular adhesion molecule-1 [ICAM-1] and E selectin), leading to rapid transmigration of leukocytes into extravascular sites.(8) While IL-1 shares many activities with TNF-α, the latter appears to represent a more attractive therapeutic target. This view is supported by observations that inhibition of TNF-α suppresses spontaneous production of IL-1, IL-6, and granulocyte-macrophage colony stimulating factor (GM-CSF) by RA synovial cells, whereas inhibition of IL-1 does not diminish expression of TNF-α.(9,10) Thus, TNFα may be a "pivotal"

cytokine in regulating expression of other inflammatory mediators in RA. This view has led to therapeutic interest in developing strategies to modulate TNF-α activity in patients with RA.

Biologic activities of TNF-α require binding to specific membrane bound TNF receptors. There are two known membrane receptors for TNF-α, designated Type I (p55 or p60)(11) and Type II (p75 or p80).(12) Receptors for TNF-α are expressed by several different cell types, including polymorphonuclear leukocytes, vascular endothelial cells and fibroblasts.(1) TNF binding to its receptors mediates a wide variety of actions, including its proinflammatory effects. Binding of sTNF p75 on RA synovial fibroblasts is increased by IL-1, IL-4, and interferon-γ (IFN-γ).(13) These same cytokines also cause an increase of TNF-α receptor (TNFR) shedding in inflammed joints.(13) Soluble TNF receptors (sTNFR) have been isolated(14,15) and demonstrated to arise from the shed extracellular portion of the membrane bound Type I (p55 or p60) and Type II (p75 or p80) molecules. Both the sTNFR p55 and sTNFR p75 have been detected in the synovial lining layers as well as deeper layers.(16) The sTNFR expressing cells are in the vicinity of TNF-α containing cells.(16) sTNF-R expressing cells have also been detected in osteoarthritis synovial tissue(17) and chondrocytes(17).

sTNFR levels are increased in sera and synovial fluid,(18-20) and can be detected at the cartilage pannus junction(21) of RA patients and patients with active systemic lupus erythematosus (SLE).(22,23) In these SLE patients, serum levels of sTNFR correlated better with disease activity than any other laboratory parameter.(22,23) There is a good correlation of the plasma levels of sTNFR p55 with levels of sTNFR p75 in patients with SLE, progressive systemic sclerosis (PSS), and mixed connective tissue disease (MCTD).(22) Both types of sTNFR's have been detected in sera of RA and OA patients even when there were no detectable levels of TNF-α.(24) Other conditions (health and disease) in which increased levels of either sTNFRp75, sTNFRp55, or both have been reported include human immunodeficiency virus (HIV) infected individuals,(25) acute

Kawasaki's disease,(26) ascites,(27) chronic renal failure,(28) hemodialysis patients,(29) hairy cell leukemia,(30) chronic lymphocytic leukemia,(30), solid tumors,(31) sepsis syndrome,(32) experimental endotoxemia,(33) transplantation,(34) cerebrospinal fluid (CSF) of multiple sclerosis (MS) patients,(35) serum of pregnant women,(36) urine of newborns,(37) amniotic fluid,(37) and meningiococcemia.(38)

The role of sTNFR's in modulating the inflammatory and immune reactions by inhibiting the effects of TNF-α are currently being investigated.(39) These two different TNF receptors mediate distinct cellular responses.(40) The p55 TNFR mediates cell cytotoxicity, whereas the p75 TNFR stimulates thymocytes proliferation.(40) The soluble forms of these receptors may play a physiologic role in protecting against the harmful effects of TNF. For example, when neutrophils adhere to the vessel wall, they release both types of sTNFR's which correlate with a decrease in neutrophil responses to TNF.(41) In vitro, sTNFR's inhibit the TNF-α mediated respiratory burst of neutrophils.(42) In persons undergoing IL-2 immunotherapy, interleukin-1 receptor antagonist (IL-1ra) and sTNFRp75 markedly down regulate IL-2 induced IL-8 synthesis.(43) IL-4, previously shown to down regulate the production of proinflammatory cytokines such as TNF-α, upregulates both types of sTNFR's on synovial cells in culture.(44) Both sTNFR p75 and sTNFR p55 inhibit the cytolytic activity of human TNF-α in vitro.(45) At low concentrations, sTNFR's may indeed stabilize TNF and augment its activity.(46) Thus, the sTNFR's may in some situations inhibit the effects of TNF, and in other situations serve as carriers for TNF and may augment the effects of TNF by prolonging its function. IL-6 may have anti-inflammatory properties by increasing circulating levels of interleukin-1 receptor antagonists (IL-1ra) and sTNFRp55.(47)

TNF-α and Animal Models

Studies in animal models of arthritis support the idea that increased TNF-α secretion plays a role in the pathogenesis of RA. Keffer, et al demonstrated that mice transgenic for the human TNF-α gene developed chronic inflammatory arthritis.(48) Treatment of these

arthritic mice with a monoclonal antibody against human TNF abrogated the arthritis. TNF-α appears to be a critical factor during the induction of collagen type II arthritis; anti-TNF-α protects against the development of arthritis.(49) When administered before the onset of disease, anti-TNFα monoclonal antibodies (MAb's) reduced the histological severity of the arthritis as well as decreasing joint swelling, while histological severity of the disease was reduced when they were given after the onset of arthritis.(50) Also, depletion of peripheral blood phagocytes with anti-rat neutrophil antiserum before passive immunization completely abolished the ability of TNF to induce arthritis.(51) More recently, combined treatment with an anti-TNF MAb and an anti-CD4 MAb in collagen-induced arthritis resulted in significantly greater reduction in pain, swelling and joint erosion than that achieved by anti-TNF MAb alone.(52) A dimeric soluble TNF receptor Fc fusion protein (rhu sTNFR:Fc) p80 was tested by Wooley et al, in mice with type II collagen-induced arthritis.(53) Mice were administered rhu TNFR:Fc before challenge with collagen; 25 percent of the treated mice developed arthritis versus 85 percent of the controls. Similarly, rhu sTNFR:Fc administered to animals that had already developed collagen-induced arthritis resulted in 40 percent improvement in the arthritis score compared to controls at 10 weeks. Piquet et al, demonstrated that both a MAb to TNF-a and the recombinant sTNFR p55 fusion protein prevented development of arthritis in DBA/1 mice immunized with type II collagen.(54)

TNF-α is most likely involved in the pathogenesis of other autoimmune disease such as MS.(53) Cerebrospinal fluid levels of TNF-α in patients with MS have been reported to correlated with the severity and progression of the neurological disease.(55) In experimental allergic encephalomyelitis (EAE), an animal model of MS, administration of an anti-TNF MAb inhibited the development of EAE.(56)

Soluble Tumor Necrosis Factor Receptor Fusion Protein

Immunex Corporation has constructed a recombinant human TNFR fusion protein (rhu sTNFR:Fc) using the Type II (p80 or p75) soluble receptor. DNA encoding the

soluble portion of the human p80 TNFR was linked to DNA encoding the Fc portion of the human IgG1, molecule and expressed in a mammalian cell line. The resulting immunoglobulin-like dimer (rhu sTNFR:Fc) possesses several attractive features as a TNF-α antagonist agent. First, the dimeric receptor fusion protein has 3000 fold higher affinity for TNF-α than the monomeric soluble receptor.(57) Second, the immunoglobulin-like Fc structure results in a longer half-life of the molecule in vivo. Finally, the immunoglobulin-like structure of rhu TNFR:Fc that may afford more rapid clearance or neutralization of the resulting complex once the molecule is bound to TNF-α.

In addition to the effects of rhu sTNFR:Fc (p80) on collagen-induced arthritis, the fusion protein has been shown to block TNF-α induced HIV expression in monocytes and lymphocytes.(58) TNF-α may play a role in the pathogenesis of Diabetes mellitus; TNF-α inhibits insulin secretion from isolated islets of Langerhans; this effect is blocked by the rhu sTNFR:Fc in a dose-dependent manner.(59) The rhu sTNFR fusion protein p75 has also been shown to suppress IL-2-induced pulmonary lymphocytic infiltration in C57BL/6 mice.(60) Both recombinant sTNFR fusion proteins p55 and p75 have been shown to prevent and treat LPS-induced lethal toxicity in mice(61,62) and intrathecal administration of endotoxin to rats.(63)

TNF-α Inhibition in Humans

Potential problems exist with the clinical use of TNF-α antagonists in RA. First, the physiologic role(s) of TNF-α is not completely understood. While TNF-α is at least partially responsible for some of the deleterious effects associated with septic shock, including increased vascular permeability, myocardial depression and intravascular coagulation, it also appears to play a beneficial role in facilitating appropriate host responses to infection. Antibodies to TNF-α as well as the rhu sTNFR:Fc fusion protein decrease the acute effects of LPS in normal subjects.(64) Preliminary studies in patients with septic shock have indicated no survival benefit for anti-TNF monoclonal antibody in treated patients compared to controls.(65,66)

Elliott et al, recently reported on 20 RA patients treated with a chimeric mouse/human monoclonal anti-TNF-α antibody, cA2.(67) The subjects, received 20 mg/kg of the MAb parenterally, and exhibited significant improvement in several clinical and laboratory measures of disease activity, including the Ritchie articular index, swollen joint count and C-reactive protein (CRP). The trial was open labeled, and not placebo controlled. A follow-up randomized, phase II, double-blind, placebo-controlled study involving 73 patients confirmed these preliminary results.(68) These trials provide further evidence for the critical role of TNF-α in the pathogenesis of RA. This same antibody is being evaluated in Crohn's disease.(69)

Experience with rhu sTNFR:Fc fusion protein (p80) in humans has been limited. Safety studies in normal human volunteers demonstrated no adverse events following intravenous administration.(70) A phase I study using rhu sTNFR:Fc (p80) in patients with severe RA was recently completed at The University of Alabama at Birmingham.(71) Sixteen patients with severe, refractory RA were treated for four weeks and observed for an additional month. All patients had failed at least one disease modifying anti-rheumatic drug (DMARD), had active disease (≥ 5 swollen joints and ≥ 9 painful joints), and were functional class I, II, or III. Concomitant treatment with an NSAID and stable (≥ 1 month) doses of prednisone (≤ 10 mg/day) were allowed. Patients were enrolled in groups of four; three in each group received active drug and one received placebo in a double-blind fashion. Rhu sTNFR:Fc was given as an intravenous load followed by 4 weeks of twice weekly subcutaneous administration. The groups were as follows:

	I.V. Loading Dose	S.Q. maintenance dose (given 2x/week x 4 weeks)
Group I:	4 mg/m^2	2 mg/m^2
Group II:	8 mg/m^2	4 mg/m^2
Group III:	16 mg/m^2	8 mg/m^2
Group IV:	32 mg/m^2	16 mg/m^2

Patient characteristics are described in Table 1. The mean age of the patients was 52.8 years (range 21 to 73), and the mean disease duration 8.5 years (range 1 to 49).

Adverse events included mild injection site rashes in 4 patients that did not necessitate discontinuation of the drug. There were no serious adverse effects and all patients completed four weeks of treatment.

Table 2 lists the major clinical parameters measured. There was no clear cut dose response among the treatment groups. Therefore, all treated patients were grouped together when analyzing the clinical response. The joint score (painful or swollen) was the total score of each joint multiplied by the severity of pain or swelling (scale of 1 to 3). At day 31, there was a 44 percent mean improvement in total pain and total joint scores in patients receiving active drug (n=12), compared to a 22 percent improvement in the patients receiving placebo (n=4). Average morning stiffness improved by 55 percent in the treated patients. Compared to baseline, there was a significant ($p < 0.05$) decrease in Westergren erythrocyte sedimentation rate (ESR) (32%). C-reactive protein (CRP) levels also decreased (27%) significantly in the treated patients compared to the placebo treated patients (13%); this was most pronounced in the highest dose group (57, 85 and 100% decrease in CRP at 31 days in the 3 patients in group IV).

Although inadequate data exists to determine whether rhu sTNFR:Fc is immunogenic, it should be noted that none of the patients in this study who received rhu sTNFR:Fc developed measurable antibody responses to the agent.

The data from this phase I dose escalation study indicate that rhu sTNF:Fc is well tolerated. There was no dose response noted with the doses used in this trial. There were trends of improvement in painful and swollen tender joint counts and biological indicators of inflammation (CRP). Efficacy of rhu sTNFR:Fc p80 in RA is being further evaluated in a multicenter phase II randomized controlled clinical trial.

Conclusions

In conclusion, considerable evidence suggests that TNF-α contributes to the pathogenesis of RA. Initial experience with a rhu sTNFR:Fc fusion protein (p80) in RA indicates the agent is well tolerated. Improvement observed in patients receiving rhu sTNFR:Fc fusion protein justifies further evaluation of this agent as a therapeutic agent in RA.

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TABLE 1

PATIENT CHARACTERISTICS AT BASELINE

Patient Number	Treatment	Dose* Group	Gender	Age	Disease Duration (yrs)	ESR (mm/hr) (Day 0)	CRP (mm/hr) (Day 0)	Swollen Joint Count (Day 0)	Tender Joint Count (Day 0)
2	Active Drug	I	F	73	8	45	5.3	26	40
3	Active Drug	I	M	50	3	25	2.6	50	64
4	Active Drug	I	F	46	5	11	1.6	7	25
5	Active Drug	II	F	21	3	35	3.5	8	9
6	Active Drug	II	M	32	5	49	3.6	38	62
7	Active Drug	II	M	60	5	25	<0.5	17	31
9	Active Drug	III	F	48	3	75	5.9	13	26
10	Active Drug	III	F	50	4	6	1.0	11	56
12	Active Drug	III	F	62	7	39	4.9	26	71
13	Active Drug	IV	F	67	2	67	4.5	19	41
15	Active Drug	IV	F	64	1	83	7.4	23	37
16	Active Drug	IV	M	54	16	47	1.8	9	25
1	Placebo	I	M	65	49	15	2.8	33	64
8	Placebo	II	F	43	2	8	<0.5	53	67
11	Placebo	III	M	47	13	35	2.5	39	55
14	Placebo	IV	F	62	15	40	3.5	20	22

*See text for dose level.

Table 2. Average percent improvement at day 31 in patients receiving rhu sTNFR:Fc or placebo.

Measurement	Active Treatment (n=12)	Placebo (n=4)
Total Painful Joint Score*	44	23
Total Swollen Joint Score*	40	25
Total Joint Score*	44	22
Morning Stiffness	55	-10
Erythrocyte Sedimentation Rate (Westergren Method)	32	12
C-Reactive Protein	27	13

* See text for descriptions on determining scores.